Ecology Research Handbook

Volume I

Liam Page

Ecology Research Handbook
Volume I

Edited by **Liam Page**

Callisto Reference
New York

Published by Callisto Reference,
106 Park Avenue, Suite 200,
New York, NY 10016, USA
www.callistoreference.com

Ecology Research Handbook: Volume I
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International Standard Book Number: 978-1-63239-159-9 (Hardback)

Printed in China.

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Volume I

Preface

The origins of the word "ecology" can be traced to the year 1866, when it was coined by the German scientist Ernst Haeckel. Ecology is an interdisciplinary field, which is the scientific study of interactions among organisms and their environment. The foundations of this subject were laid by Ancient Greek philosophers such as Hippocrates and Aristotle, who studied natural history. From the 19th century onwards, concepts such as natural selection and adaptation transformed Ecology into a more rigorous discipline.

The concepts of Ecology are premised on ecosystems, which include organisms, the communities they make up, and the non-living components of their environment. The scope of Ecology spans a wide array of interacting levels of organisms, ranging from the micro-level to planetary scale. This subject helps comprehend how the living world interacts. It provides evidence on the interdependence between the inanimate and animate elements of the ecosystem.

Ecology also covers topics of subjects like Biology and Earth Science. Topics of interest to ecologists include diversity, distribution, and population of organisms. The manner in which biodiversity affects ecological function is an important and emerging focus area in ecological studies. A better understanding of ecological systems is crucial in contemporary times, as it allows scientists to predict the consequences of human activity on the environment.

I would like to thank our researchers and writers for sharing their valuable research with us in this book.

Editor

Contents

Plants used in traditional beekeeping in Burkina Faso

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ABSTRACT

Beekeeping is one of the recommended approaches in the implementation of poverty alleviation programs in rural areas of Burkina Faso. However, plants that are important in beekeeping have not been identified. The use of parts and organs of plants by beekeepers and their methods of harvesting remain unknown. These limit the conservation efforts of these important plants and affect beekeeping development. The study was carried out in the south-central, east-central regions and in Comoé and Boucle of Mouhoun regions of Burkina Faso. The objective of the study was to identify the plants species used by traditional beekeepers, the different uses made of these plant parts and organs and then to discuss the impact of these activities on the survival of the plant resources. An ethnoapiculture survey was conducted in the main apiculture zone of Burkina Faso, using semi-structured interviews. The methodology of botanical coherence or convergence was applied to classify botanical species. Results showed that 35 botanical species were used in traditional beekeeping. The use of plant parts or organs in traditional hives construction represents 55%, attraction of wild swarms in new beehives is 37.50% and use as a torch or as a smoker, 7.50%. The barks are the organs most used. Trees are botanical type most used. The results are not exhaustive and therefore other additional studies need to be carried out. In order to sustain the use of these important plants, their growing in nursery and their planting in the field are recommended.

Keywords: Beekeeping; Melliferous Plants; Pollinating; Biodiversity; Burkina Faso

1. INTRODUCTION

Honeybees are since 2006 victims of colony collapse disorder or (CCD) ([1-3]). Many well intentioned suggestions as to the possible causes of colony losses, including such improbable ideas as mobile telephones, genetically modified crops and nanotechnology, have perhaps overshadowed much more likely explanations such as pests and diseases, pesticides, loss of forage and inappropriate beekeeping practices [2]. Bees are the major pollinators of wild plants and crops in terrestrial ecosystems. Honeybees are known to contribute significantly to the provision of this essential ecosystem service of pollination [4-6]. They are also bio-indicators for environment pollution [7,8] and beekeeping is an effective means to generate monetary incomes that support the livelihood of rural communities. Numerous studies have demonstrated the economic value of honeybees to the agricultural industry of the world [9,10]. In Africa, especially areas in south of Sahara and particularly in Burkina Faso, this phenomenon is not fully known because of the lack of scientific studies [11]. [6] stated that beekeepers and honey hunters are sometimes perceived to cause damages to forests, through the careless use of fire during harvesting and because they kill trees to make beehives. So, traditional beekeeping has been considered as harmful to biodiversity conservation [12]. Others authors differentiate traditional beekeeping from honey hunting as contributing to the increase in honey bee number. The roles of honeybees in providing ecosystem services is a function of their number in the beehive and varies according to the type of beehive used [13].

Burkina Faso has undertaken the modernization of its apiculture since 1987. Studies had been done on the melliferous plants [14,15] and on the plant organs used to attract swarms of the local honeybee *Apis mellifera adansonii* Latreille into newly installed beehives [16]. Traditional beekeeping is widespread in Burkina Faso and their activities understand in the exploitation of plant parts and organs described as "extractivism" may have conservation undertones [17]. Studies on the impact of this activity on colony loss and other effects on the environment have not been carried out. The harvest technologies of plant organs or parts remain unrecognized, limiting the conception of preservation efforts of melliferous plants. This lack of information can moreover constitute a handicap in the development of beekeeping. It is to contribute to raising this constraint in relationship with the lack of information that the present study aims to provide knowledge on the used in traditional beekeeping. It will identify the various uses of the plant parts and organs and discuss their impacts on plant resources sustainability and then suggest solutions for a sustainable management of the identified plants.

2. MATERIAL AND METHODS

2.1. Study Area

The study was carried out in the villages of Nazinga (south-central region), Garango (east-central region), Tiefora (Comoé region) and Dédougou (Boucle of Mouhoun region) (**Figure 1**).

These communities fall within the main beekeeping zones made up of the north and south Soudanian phytogeographical sector of Burkina Faso.

Agriculture (crop cultivation and animal breeding) is the main activity of the population. In all the regions, crops (*Sorghum guineense* Staph., *Zea mays* Linn., *Oryza sativa* Linn., *and Dioscorea dumetotum* (Kunt) Pax.) are dominant.

The vegetation is predominantly savannas with arustic-landscapes dominated by species such *Vitellaria paradoxa* Gaertn, *Tamarindus indica* Linn., *Parkia biglobosa* (Jacq.) Benth, *Lannea microcarpa* Engl. & K. Krause, *Adansonia digitata* Linn., *Faidherbia albida* (Del.) A. Chev.; the groupings of *Anogeissus leiocarpus* (DC.) Guil. & Perr. and planted species as *Mangifera indica* Linn., *Eucalyptus camaldulensis* Dehnhard, *Azadirachta indica* A. Juss, *Khaya senegalensis* (Desn.) A. Juss, *Anacardium occidentale* Linn., *Borassus aethiopum* Mart., *Psidium guajava* Linn., *Cariaca papaya* Linn., *Annona squamosa* Linn., and *Citrus sinensis* (Linn.) Osbeck.

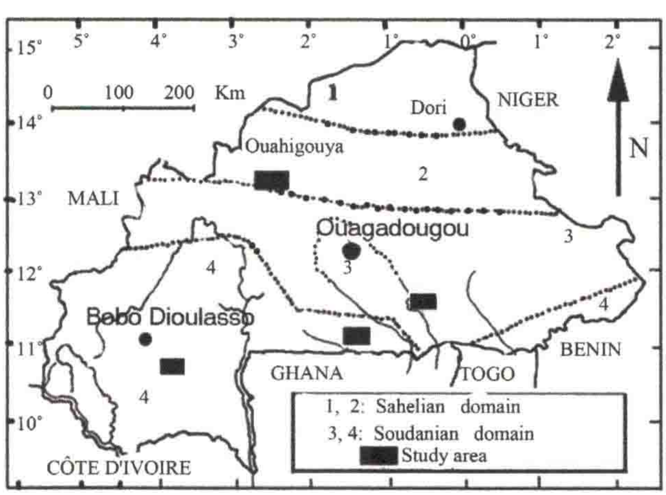

Figure 1. Location of the study area.

2.2. Methods

Ethno-apicultural investigations and field observations were carried out using semi-structured inquiry cards on traditional beekeepers who are 25 years of age and possessing colonized traditional beehives. The names of plants used were transcribed into the following local languages: Gourounsi for Nazinga zone; Bissa for Garango zone and Dioula for Tiéfora and Dédougou zones. The plant species scientific identification was made referring to [18]. The plants parts and organs used by beekeepers were identified from responses obtained from at least 10 beekeepers. A total of 103 beekeepers were interviewed.

3. RESULTS

3.1. Different Plant Parts or Organs Used

The results showed that the barks and fibers with 37.5% of utilizations constituted the most organs used (**Figure 1**). The grass, the aerial organs, the thatches of graminaceous and the inflorescences constituted the group of plants aerial part (32.5%). The twigs constituted 12.5%, the fruits and seeds 10%, leave 5% and the tubers were less used (2.5%).

3.2. Plants Used and Their Utilizations

The parts or organs of 35 botanical species were used in the traditional beekeeping practices in Burkina Faso (**Table 1**).

Three kinds of utilization of the plant parts or organs were dominants (**Table 1**). The first concerned the use in the new traditional beehives construction. It repre-

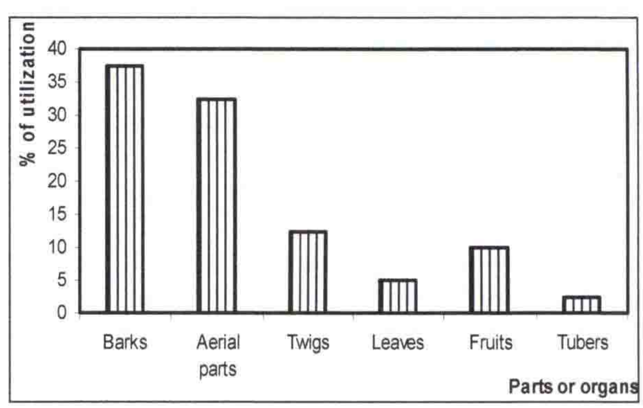

Figure 2. Different plant parts or organs used in traditional beekeeping.

sented 55%. Indeed, beehives can be made with barks, hollowed out tree trunks, plaited straws or twigs.

The second represented the attraction of wild swarms into newly established beehives. It represented 37.50%. Indeed, plant parts or organs can be used as swarm baits substituting the Aristée perfumes, the honeybees' charm or comb foundations used in modern beekeeping.

The third is the use as a torch to light beehives inside or as a smoker during honey harvesting. It represented 7.50%.

3.3. Botanical Type Used

In the biological type, trees were the most used (44%); followed in order by the grass (31%), the shrubs (22%) and the lianas (3%) (**Figure 3**).

Table 1. The use of plant parts and organs in traditional beekeeping in the Burkina Faso.

Scientifique names	Parts or organs used	Utilizations
Acacia seyal Del.	Fruits	Swarms attraction
Combretum glutinosum Perr. ex DC	Twigs	Swarms attraction
Ctenium newtonii Hack.	Thatches	Swarms attraction
Cymbopogon schoenanthus subsp. *proximus* (Hochst. ex A. Rich.) M. & W	Inflorescences	Swarms attraction
Dicoma tomentosa Cass.	Aerial organs	Swarms attraction
Dioscorea dumetorum (Kunth) Pax	Tubers	Swarms attraction
Diospyros mespiliformis Hochst. ex A. DC.	Leaves	Swarms attraction
Guiera senegalensis J. F. Gmel.	Twigs	Swarms attraction
Hyptis spicigera Lam.	Aerial organs	Swarms attraction
Leucas martinicensis (Jacq.) Ait.	Aerial organs	Swarms attraction
Ocimum americanum Linn.	Aerial organs	Swarms attraction
Parkia biglobosa (Jacq.) Benth.	Seeds	Swarms attraction
Piliostigma reticulatum (DC.) Hochst.	Fruits	Swarms attraction
Piliostigma thonningii (Schum.) Milne-Redhead	Fruits	Swarms attraction
Andropogon ascinodis C. B. Clarke	Thatches	Honey harvest
Andropogon gayanus Kunth	Thatches	Honey harvest
Andropogon pseudapricus Stapf	Thatches	Honey harvest
Andropogon ascinodis C. B. Clarke	Thatches	Beehive construction
Andropogon gayanus Kunth	Thatches	Beehive construction
Andropogon pseudapricus Stapf	Thatches	Beehive construction
Borassus aethiopum Mart.	Leaves	Beehive construction
Burkea africana Hook.	Bark	Beehive construction
Daniellia oliveri (Rolfe) Hutch. & Dalz.	Bark	Beehive construction
Detarium microcarpum Guill. & Perr.	Bark	Beehive construction
Feretia apodanthera Del.	Twigs	Beehive construction
Hibiscus asper Linn.	Fibers	Beehive construction
Isoberlinia doka Craib & Stapf	Bark	Beehive construction
Lannea acida A. Rich.	Fibers	Beehive construction
Loudetia togoensis (Pilger) C. E. Hubbard	Thatches	Beehive construction
Piliostigma reticulatum (DC.) Hochst.	Fibers	Beehive construction
Piliostigma thonningii (Schum.) Milne-Redhead	Fibers	Beehive construction
Prosopis africana (Guill. & Perr.) Taub.	Bark	Beehive construction
Pseudocedrela kotschyi (Schweinf.) Harms	Bark	Beehive construction
Pterocarpus erinaceus Poir.	Bark	Beehive construction
Saba senegalensis (A. DC.) Pichon	Twigs	Beehive construction
Fluggea virosa (Roxb. ex Willd.) Baill	Twigs	Beehive construction
Tamarindus indica Linn.	Fibers	Beehive construction
Terminalia avicennioides Guill. & Perr	Bark	Beehive construction
Vitellaria paradoxa Gaertn.	Bark	Beehive construction
Xeroderris stuhlmannii (Taub.) Men	Bark	Beehive construction

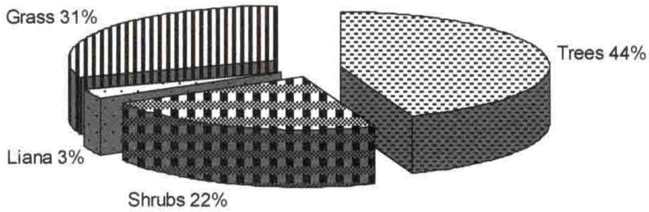

Figure 3. Different plants biological types used in traditional beekeeping.

4. DISCUSSION

In the traditional beekeeping practices, different plants, parts or organs are used by the beekeepers in different ways. The construction of beehives is more important in the utilization of plants and also their parts or organs. The technology used to remove these parts or organs can be negative for the environment because it affects the regeneration and the survival of the plants used. Indeed, according to [17], the cutting down has negative impacts on the individual tree, because, even if it presents a potential of stump rejections, they have only very slight chance to survival. That will appear as habitat degradation and outright destruction and can be the major causal factor in the decline of bees [5].

Often, the plants used were also excellent nectar species and then the loss of trees has negative implications for beekeepers because they lose food and nesting sites for wild bees, materials for building hives and places to keep hives. However, beekeepers must make deliberate and conscious efforts to protect and conserve forests in which their bees forage, despite their dependency on these resources.

There are also positives impacts on traditional beekeeping practices. Indeed, nesting honeybees in appropriate way allows them to increase their number that will increase their role (pollinating, honey production). Often, the traditional beekeepers breed their honeybees even if the hives used are rudimentary; and they only use the smoke of burned thatch to hunt honeybees during the harvest [16]. According to [17], the removal or whatever organ collected is mostly made to secure not only the survival of the exploited individual, but also the regeneration of the resource in a reasonable lapse of time. Furthermore, according to [6], the development of traditional beekeeping based on keeping colonies, to the detriment of the honey hunting can increase the honeybees' number per beehive and even per region, involving thus an increase of their pollinating role. This development minimizes the destructive effects of traditional beekeeping on honeybees on one hand and the environment on the other. Also, according to [16], the utilization of plant organs or parts to attract honeybee swarms in newly established beehives contributes to reduce the installation costs of beekeeping projects development.

According to [6], the technologically modern man has contributed to honeybees declines in Africa. This is evident in that bee diversity and abundance is much greater on crops in areas surrounded by natural vegetation than in ecosystems that have been widely transformed by agriculture and other exotics along with removal of natural vegetation through urbanization.

5. CONCLUSION

Traditional beekeeping contributes greatly to biodiversity conservation in Burkina Faso. Despite the negative effects that are attributed to some of its activities, it allows for the establishment and management of wild swarms of honeybees in appropriated ways for hive products. The pollinating role of honeybees in the ecosystem is therefore enhanced. Plants, parts and organs are used at different levels in this system of apiculture. The effects of the use of plants and their organs in traditional beekeeping practices on the vegetation and the environment remain negligible. Traditional beekeepers therefore sustain the populations of honeybees in the environment which contribute to the essential ecosystem service of pollination and biodiversity conservation. Negative practices of wild honey hunting should be replaced with traditional beekeeping.

REFERENCES

[1] Chagnon, M. (2008) Causes et effets du déclin mondial des pollinisateurs et les moyens d'y remédier. Fédération Canadienne de la Faune. Bureau régional du Québec.

[2] Neumann, P., and Carreck, N.L. (2010) Honey bee colony losses. *Journal of Apicultural Research*, **49**, 1-6.

[3] Kluser, S., Neumann, P., Chauzat, M.-P. and Pettis, J.S. (2010) Global honey bee colony disorder and other threats to insect pollinators. UNEP.

[4] Vaissière, B., Morison, N. and Carré, G. (2005) Abeilles, pollinisation et biodiversité. *Abeilles & Cie*, **3**, 10-14.

[5] Brown, M.J.F. and Paxton, R.J. (2009) The conservation of bees: A global perspective, *Apidologie*, **40**, 410-416.

[6] Eardley, C.D., Gikungu, M. and Schwarz, M.P. (2009) Bee conservation in Sub-Saharan Africa and Madagascar: Diversity, status and threats, *Apidologie*, **40**, 355-366.

[7] Chauzat, M.-P., Faucon, J.-P., Martel, A.-C., Lachaize, J., Cougoule, N. and Aubert, M. (2006) Les pesticides, le pollen et les abeilles LSA, **216**, 11-12.

[8] Bogdanov, S. (2006) Contaminants of bee products. *Apidologie*, **37**, 1-18.

[9] Krell, R. (1996) Value-added products from beekeeping. FAO Agricultural Services Bulletin No. 124.

[10] Bradbear, N. (2010) Le rôle des abeilles dans le dévelop-

pement rural. Manuel sur la récolte, la transformation des produits et services dérivés des abeilles. FAO, Rome, PFNL 19.

[11] Dietemann, V., Pirk, C.W.W. and Crewe, R. (2009) Is there a need for conservation of honeybees in Africa? *Apidologie*, **40**, 285-295.

[12] Fisher, F.U. (1993) L'élevage des abeilles dans l'économie de base de la savane arborée de Miombo au Centre de l'Afrique Australe. *Document du Réseau Forestier Pour le Développement Rural*, **15**, 3-11.

[13] Foucault, B. (2010) Ethonographie de quelques ruches traditionnelles. *Colloque sur les Journées d'échanges sur l'abeille et l'apiculture*, 22-27 Avril 2005, Lille, 121-128

[14] Guinko, S., Guenda, W., Tamini, Z. and Zoungrana, I. (1992) Les plantes mellifères de la zone Ouest du Burkina Faso. *Etudes flor. Vég. Burkina Faso*, **1**, 27-46.

[15] Nombré, I., Schweitzer, P., Sawadogo, M., Boussim, J.I. and Millogo-Rasolodimby, J. (2009) Assessment of melliferous plant potentialities in Burkina Faso. *African Journal of Ecology*, **47**, 622-629.

[16] Nombré, I., Schweitzer, P., Boussim, I.J., Millogo/Rasolomdimby, J. (2009) Plantes utilisées pour attirer les essaims de l'abeille domestique (*Apis mellifera adansonii* Latreille) au Burkina Faso. *International Journal of Biological and Chemical Sciences*, **3**, 840-844.

[17] Aubertin, C., De Castro, A.L., Empenire, L., Lescure, J.-P., Mitja, D. and Pinton, F. (1993) Les activités extractivistes en Amazonie Centrale: Une première synthèse d'un projet multidisciplinaire. ORSTOM/INPA.

[18] Arbonnier, M. (2002) Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. CIRAD, MNHN, UICN.

2

Parental care in the freshwater crab *Sylviocarcinus pictus* (Milne-Edwards, 1853)*

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ABSTRACT

Parental care is a common strategy in many animal groups, to increase survival of the offspring. Here, we report parental care in the freshwater crab *Sylviocarcinus pictus*. A female caught in the Amazon River, Brazil, bore juvenile crabs rather than eggs on her abdomen. Kept in the laboratory, the female retained the juveniles on the abdomen for 17 days, after which the juveniles left the abdomen. A total of 341 juvenile crabs measuring 3.45 ± 0.12 mm were recorded. This pattern of parental care is very important for the maintenance of local populations of *S. pictus*, because if the larvae were released, as occurs in many marine species, they would drift downstream.

Keywords: Freshwater Crab; Parental Care; Reproduction; Trichodactylidae

1. INTRODUCTION

In many animal species, parental care is a common reproductive strategy [1-3]. The many patterns of parental care include biparental, or uniparental by either males or females [1]; that may show manipulation of sex differences in parental care [4] and is energetically costly [5]. Many groups of invertebrates show some form of parental care. For instance, in insects the most rudimenttary form of maternal care is provided by females that incorporate toxins into their eggs, oviposit them in protected places, or cover their eggs with a hard wax-like shell before abandoning them [3]. In arachnids, females of *Bourguyia albiornata* Mello-Leitão 1923 oviposit almost exclusively inside the tube formed by the curled leaves of the bromeliad *Aechmea nudicaulis* (Linnaeus) Grisebach, 1864 [6]. Jawed Hirudinidae deposit desiccation-resistant cocoons on land and many species brood the eggs and young [7].

In decapod crustaceans, parental care is usually restricted to females that carry the eggs in the brood compartment, and care is terminated when the larvae are released into the plankton [8,9]. However, among other examples in crustaceans, [10] presented evidence of a direct link between active brood care and provision of oxygen to the young. For amphipods, [11,12] studied active maternal brooding and juvenile care. The preparation of a nest structure to defend and feed its young in the crab *Metopaulias depressus* Rathbun, 1918 was recorded [9,13].

For freshwater crustaceans, available information about parental care is sparse in comparison with marine species. The species that has been most studied is the crab *M. depressus*, in several aspects, *i.e.*, parental care in an unusual environment [13], protection of larvae from predation by damselfly nymphs [9], maintaining oxygen, pH and calcium levels optimal for the larvae [14] and evolution theory [15]. In freshwater crabs, 15 species have been reported to bear juvenile crabs attached to the female abdomen [16]. The extended brood care was reported in species of all five families of primary freshwater crabs [17]. Here, we record a female of the freshwater crab *Sylviocarcinus pictus* (Milne-Edwards, 1853) with juvenile crabs attached on the abdomen, indicating the existence of parental care in this species.

2. MATERIAL AND METHODS

The female of *S. pictus* was collected by hand, on a bank of the Amazon River (03°08'13.7"S; 58°27'46.8"W) in October 2011 (**Figure 1**). The specimen was placed in a plastic box with aerated water and transported to the laboratory. In the laboratory, the carapace width was

*Research Group on Biology and Production of Amazonic Aquatic Organisms measured with a caliper (0.05 mm), and the crab was maintained in an aquarium for 17 days.

3. RESULTS AND DISCUSSIONS

This female had a carapace width of 41.5 mm, with 341 juvenile crabs attached to the abdomen (**Figure 2**). The mean size of the juveniles was 3.45 ± 0.12 mm, ranging from 3.10 to 3.66 mm. The juveniles remained on the female's abdomen for 17 days; during this period, they would occasionally leave the female's abdomen for several minutes. After 17 days, all the juveniles permanently left the female's abdomen. *Sylviocarcinus pictus* shows gregarious behavior, and the juveniles can cling to their mother, as also observed in the freshwater crab *Potamon edulis* (*P. fluviatile*) (Latreille, 1818) by [18], this kind of behavior is common for freshwater crabs. According to [20], the juveniles of the potamid crab *Candidiopotamon rathbunae* (de Man, 1914) are essentially independent after their first day of life, but often return to the mother for shelter during the following 2 weeks. The same pattern was observed for the juveniles of *S. pictus*.

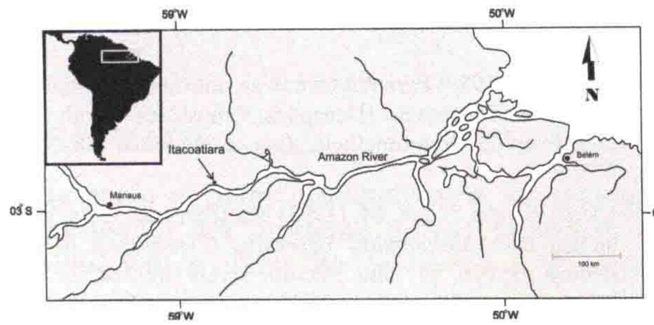

Figure 1. Location of the study area, Itacoatiara (arrow) on the Amazon River, Brazil.

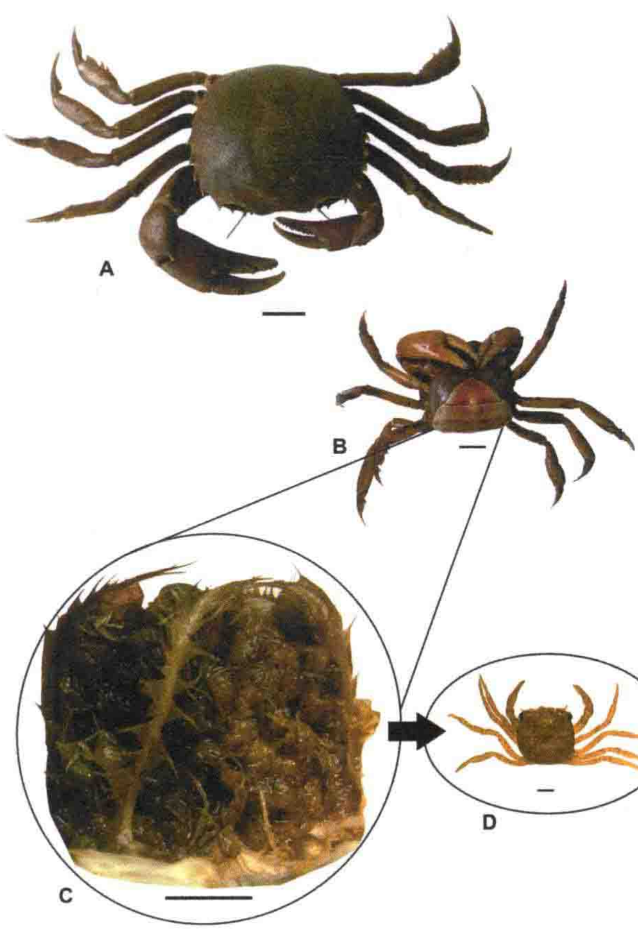

Figure 2. Dorsal view of *Sylviocarcinus pictus* (A), ventral view of *S. pictus* (B), detail of juvenile crabs attached on the female abdomen (C) and juvenile crab (D). Scale bar of the figures A, B and C = 10 mm, and of figure D = 1 mm.

In a recent study [16] observed two size groups of juvenile crabs with different carapace morphology, attached on the abdomen of females of the crab *Kingsleya ytupora* Magalhães, 1986, suggesting that the juveniles are attached to females for a prolonged period. In the present study, all juveniles had the same morphology and similar size, and remained on the female's abdomen for 17 days in the laboratory. However, as recorded by [16], we did not observe the hatching process and cannot accurately report the full period of juvenile incubation by females.

According to [11], brood care is called "active" if specific parental activities are directed toward the brood, and "passive" if such specific behavior is lacking. Females of *S. pictus* could be considered "active" in parenmtal activities, since in this freshwater crab the embryonic and larval periods are completed entirely in the egg stage, resulting in hatching of miniature adults [20]; these are considered juveniles, and remain on the abdomen.

Abbreviated larval development is often accompanied by increased parental care. According to [21], in its broadest sense, parental care includes preparation of nests and burrows, production of heavily yoked eggs, care of the eggs, provisioning of the young, and care of the offspring after they reach nutritional independence. Parental care significantly affects the ecological success and evolutionary potential of species by enhancing the survival and fitness of the offspring. In the freshwater caridean shrimp *Dugastella valentina*, [22] observed both the abbreviated development and parental safeguarding until the decapodid stage obviously reduce the risk of being washed away or of being predated upon. Simultaneously, this type of parental care could mean a limited gene flow and hence a high degree of genetic divergence between populations, because of the low dispersal ability of the larvae [22]. In populations of *S. pictus*, the epimorphic development and parental care could produce a similar situation.

This pattern is common in primary freshwater crabs, because in freshwater habitats there are strong selective pressures toward reduction in egg number and increase in egg size, abolishment of free larvae, and extension of brood care until the juvenile stage, resulting in a marked reduction in dispersal and gene flow, and leading to the high degree of endemism and speciation seen in these crustaceans [17]. However, under conditions of rapid habitat destruction, environmental pollution and global warming, slow dispersal of direct developers may become a severe disadvantage, impairing replacement of lost populations and placing the directly developing taxa at a greater risk of extinction than the indirectly developing taxa [17].

4. CONCLUSIONS

The present study records extended parental care in the crab *S. pictus*, contributing to knowledge of the reproduction of freshwater crabs. This pattern of parental care is very important for the maintenance of local populations of *S. pictus*, because if the larvae were released, as occurs in many marine species, they would drift downstream.

REFERENCES

[1] Webb, J.N., Houston, A.I., McNamara, J.M. and Székely, T. (1999) Multiple patterns of parental care. *Animal Behaviour*, **58**, 983-993.

[2] Cockburn, A. (2006) Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society B*, **273**, 1375-1383.

[3] Scott, M.P. (2009) Parental care. In: Resh, V.H. and Cardé, R.T., Eds., *Encyclopedia of Insects*, 2nd Edition, Academic Press, New York, 751-753.

[4] Wring, J. and Cuthill, I. (1989) Manipulation of sex differences in parental care. *Behavioral Ecology and Sociobiology*, **25**, 171-181.

[5] Lardies, M.A., Cotoras, I.S. and Bozinovic, F. (2004) The energetics of reproduction and parental care in the terrestrial isopod *Porcellio laevis*. *Journal of Insect Physiology*, **50**, 1127-1135.

[6] Machado. G. and Oliveira, P. (2002) Maternal care in the neotropical harvestman *Bourguyia albiornata* (Arachnida; Opiliones): Oviposition site selection and egg protection. *Behaviour*, **139**, 1509-1524.

[7] Kutschera, U. and Wirtz, P. (2001) The evolution of parental care in freshwater leeches. *Theory in Bioscience*, **120**, 115-137.

[8] Hazlett, B.A. (1983) Parental behaviour in decapod Crustacea. In: Rebach, S. and Dunham, D.W., Eds., *Studies in Adaptation. The Behaviour of Higher Crustacea*, John Wiley, New York, 171-193.

[9] Diesel, R. (1992) Maternal care in the bromeliad crab, *Metopaulias depvessus*: Protection of larvae from predation by damselfly nymphs. *Animal Behaviour*, **43**, 803-812.

[10] Baeza, J.A. and Fernández, M. (2002) Active brood care in *Cancer setosus* (Crustacea: Decapoda): The relationship between female behaviour, embryo oxygen consumption and the cost of brooding. *Functional Ecology*, **16**, 241-251.

[11] Dick, J.A., Faloon, S.E. and Elwood, R.W. (1998) Active brood care in an amphipod: Influences of embryonic development, temperature and oxygen. *Animal Behaviour*, **56**, 663-672.

[12] Thiel, M. (1998) Extended parental care in marine amphipods. I. Juvenile survival without parents. *Journal of Experimental Marine Biology and Ecology*, **227**, 187-201.

[13] Diesel, R. (1989) Parental care in an unusual environment: *Metopaulias depressus* (Decapoda: Grapsidae), a crab that lives in epiphytic bromeliads. *Animal Behaviour*, **38**, 561-575.

[14] Diesel, R. and Schuh, M. (1993) Maternal care in the bromeliad crab *Metopaulias depressus* (Decapoda): Maintaining oxygen, pH and calcium levels optimal for the larvae. *Behaviour Ecology and Sociobiology*, **32**, 11-15.

[15] Schubart, C. D, Diesel, R. and Hedges, S. B. (1998) Rapid evolution to terrestrial life in Jamaican crabs. *Nature*, 393, 363-365.

[16] Wehrtmann, I.S., Magalhães, C., Hernáez, P. and Mantelatto, F.L. (2010) Offspring production in three freshwater crab species (Brachyura: Pseudothelphusidae) from the Amazon region and Central America. *Zoologia*, **27**, 965-972.

[17] Vogt, G. (2013) Abbreviation of larval development and extension of brood care as key features of the evolution of freshwater Decapoda. *Biological Reviews*, **88**, 81-116.

[18] Pace, F., Harris, R.R. and Jaccarini, V. (1976) The embryonic development of the Mediterranean freshwater crab, *Potamon edulis* (*P. fluviatile*) (Crustacea, Decapoda, Potamonidae). *Journal of Zoology*, **180**, 93-106.

[19] Liu, H.C. and Li, C.W. (2000) Reproduction in the freshwater crab *Candidiopotamon rathbunae* (Brachyura: Potamidae) in Taiwan. *Journal of Crustacean Biology*, **20**, 89-99.

[20] Von Sternberg, R., Cumberlidge, N. and Rodriguez, G. (1999) On the marine sister groups of the freshwater crabs (Crustacea: Decapoda: Brachyura). *Journal of Zoological Systematics and Evolutionary Research*, **37**, 19-38.

[21] Clutton-Brock, T.H. (1991) The evolution of parental care. Princeton University Press, Princeton, 1-352,

[22] Cuesta, J.A., Palacios-Theil, E., Drake, P. and Rodríguez, A. (2006) A new rare case of parental care in decapods. *Crustaceana*, **79**, 1401-1405.

Biodiversity and secretion of enzymes with potential utility in wastewater treatment

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ABSTRACT

The main organic contaminants in municipal wastewater are proteins, polysaccharides, and lipids, which must be hydrolyzed to smaller units. A high concentration of oil and grease in wastewater affects biological wastewater treatment processes by forming a layer on the water surface, which decreased the oxygen transfer rate into the aerobic process. Microbial proteases, lipases, amylases, and celullases should play essential roles in the biological wastewater treatment process. The present study aimed to isolate lipase- and other hydrolytic enzyme-producing microorganisms and assess their degradation capabilities of fat and oil wastewater in the laboratory. We also evaluated microbial interactions as an approach to enhance lipolytic activity. We place emphasis on lipase activity because oil and grease are not only environmental pollutants, but also form an undesirable tough crust on pipes of sewage treatment plants. Thirty-five lipolytic microorganisms from sewage were identified and assessed for hydrolytic enzyme profiles. Lipases were characterized in detail by quantification, chain length affinity, and optimal conditions for activity. The good stability of isolated lipases in the presence of chemical agents, thermal stability, wide range of pH activity and tolerance, and affinity for different lengths of ester chains indicates that some of these enzymes may be good candidates for the hydrolysis of organic compounds present in wastewater. A combination of enzymes and fermenting bacteria may facilitate the complete hydrolysis of triglycerides, proteins, and lignocellulose that normally occur in the wastes of industrial processes. This study identifies enzymes and microbial mixtures capable of digesting natural polymeric materials for facilitating the sewage cleaning process.

Keywords: Lipase; Esterase; Wastewater; Sewage Treatment; Lipolytic Microorganisms; Amylase; Protease; Cellulase

1. INTRODUCTION

For more than a century, biological wastewater treatment has been used to minimize anthropogenic damage to the environment. Oil and Grease (O&G) are the major problems and contaminants in biological wastewater treatment processes. Because of their nature, O&G form a layer on the water surface and decrease the oxygen transfer rate into an aerobic process [1]. These contaminants are mainly discharged from restaurants, food industries, and households [1,2]. Proteins and polysaccharides must also be hydrolyzed to smaller units by extracellular enzymes in a municipal wastewater treatment plant [3,4].

The composition and activity of the microbial community within a wastewater treatment plant play a substantial role in the efficiency and robustness of the purification process [5]. The efficiency of conventional biological processes in wastewater treatment is reduced by the high concentrations of O&G in effluents [6]. In the activated sludge process, high levels of O&G lead to a reduction of biological activity of the flocs due to the difficulty of oxygen and substrate to penetrate the floc due to the oil film formation around it [7]. Moreover, in the case of anaerobic digestion, excessive amounts of O&G inhibit the action of acetogenic and methanogenic bacteria [6,8,9]. The Brazilian National Council on the Environment (CONAMA) established the maximum level of mineral oil concentration allowed for effluent in water bodies at 20 mg/l, and the maximum level of vegetable oils and animal fats to 50 mg/l in Article 34, Resolution number 357 established on March 17, 2005 [10].

Traditional approaches to treat oily effluents include gravity separation, dissolved air flotation (DAF), de-emulsification, coagulation, and flocculation. Free oil is removed from wastewater by gravity separation; however, this process cannot remove small oil droplets and emulsions. Oil that adheres to the surface of solid particles can be removed by particle settling [11]. DAF uses solubilized air to increase buoyancy of the smaller oil droplets and improve separation. In addition, emulsified oil is removed by chemical or thermal de-emulsifying processes, or both [11]. Wastewater containing emulsified oil is heated to reduce the viscosity, accentuate density differences, and weaken the interfacial films stabilizing the oil phase. Thereafter, acidification and the addition of a cationic polymer neutralize the negative charges, and elevation of pH to an alkaline level induces flocculation of inorganic salts. Flocs with adsorbed oil are separated and the sludge is dewatered [11]. In this context, the usage of lipolytic microorganisms in wastewater treatment could eliminate this pretreatment process [12]. Chigusa *et al.* [13] showed that the percentage of fat in wastewater treated with a mixed culture of nine lipase-producing yeast strains decreased by 94%.

Oily effluents can also be pretreated as an approach to conform with the CONAMA resolution, but the achievement of such pretreatment processes depends on the costs of the enzyme [14]. Many industrial processes require breakdown of solids and the prevention of fat blockage or filming in waste systems before the wastewater can be released into the sewage system. This can be accomplished 1) by degradation of organic polymers with a commercial mixture of lipase, cellulase, protease, amylase, and inorganic nutrients; or 2) by sewage treatment, cleaning of holding tanks, septic tanks, grease traps, and other systems. WW07P is produced by Environmental Oasis Ltd. and contains a range of high-performance microorganisms adapted for use in the biological treatment of wastewater containing high fat and oils. It also contains surfactants capable of liquefying heavy fat deposits, thereby assisting in their biodegradation [15].

Lipases and esterases constitute a large category of ubiquitous enzymes expressed by many organisms. Carboxylesterases (EC 3.1.1.1) have broad substrate specificity toward esters and thioesters. Esterases that hydrolyze long-chain acylglycerols (containing more than 10 carbon atoms) are termed lipases (EC 3.1.1.3) and can be considered lipolytic and esterolytic enzymes [16]. Most lipases are water-soluble enzymes that hydrolyze ester bonds of water-insoluble substrates [17]. Therefore, lipases act at the interface between a substrate phase and an aqueous phase, in which the enzyme is dissolved [18]. It is often necessary to combine two or more lipases in order to release a glycerol molecule, since all three acyl chains of a triacylglycerol molecule are rarely released by a single lipase [17].

The α-amylases (E.C.3.2.1.1) are enzymes that hydrolyze starch molecules to generate progressively smaller polymers composed of glucose units [19]. Today, a large number of microbial amylases have almost completely replaced the chemical hydrolysis of starch. The main advantage of using microorganisms for the production of amylase is the ability to bulk produce the enzyme and the easy manipulation of microbes to achieve enzymes with desired characteristics. Moreover, the stability of microbial amylases are higher than those of plant and animal [20].

Microbial proteases are used in waste treatment from various food-processing industries and household activities to solubilize proteinaceous waste and reduce the biological oxygen demand of aquatic systems [21,22]. Hydrolytic enzymes, such as lipases, amylases, and proteases, have a promising application in wastewater treatment of candy, ice cream, dairy, and meat industries. Enzymes for wastewater treatment do not require purification and thus should present a low production cost [23]. These characteristics have led to an increasing interest in enzyme production technology and the search for new microorganisms with a diverse ability to produce enzymes [24-27].

Microbial cellulases are widely used in the paper, wine, animal feed, and textile industries as well as for biofuels production, food processing, olive oil and carotenoid extraction, and waste management [28]. The wastes generated from agricultural fields and agroindustries contain a large amount of unutilized cellulose, thereby causing environmental pollution. Today, these wastes are utilized to produce valuable products, such as enzymes, sugars, biofuels, chemicals, and others [29-33].

Biosurfactants are amphiphilic molecules that possess both polar and nonpolar domains that have effective surface-active properties. There are two main types of these molecules: 1) those that reduce surface tension at the air-water interface (biosurfactants), and 2) those that reduce the interfacial tension between immiscible liquids or at the solid-liquid interface (bioemulsifiers) [34,35]. Biosurfactants usually exhibit an emulsifying capacity, but bioemulsifiers do not necessarily reduce the surface tension [34,35]. These molecules are microbial synthesized, and the different types of biosurfactants include lipopeptides synthesized by many species of *Bacillus*, glycolipids synthesized by *Pseudomonas* and *Candida* sp., phospholipids synthesized by *Thiobacillus thiooxidans*, and polysaccharidelipid complexes synthesized by *Acinetobacter* sp. [36-38].

The emulsification of lipids through the breakdown of lipid droplets favors the occurrence of hydrolysis, since the water-soluble lipolytic enzymes have greater surface contact with the substrate to be hydrolyzed.